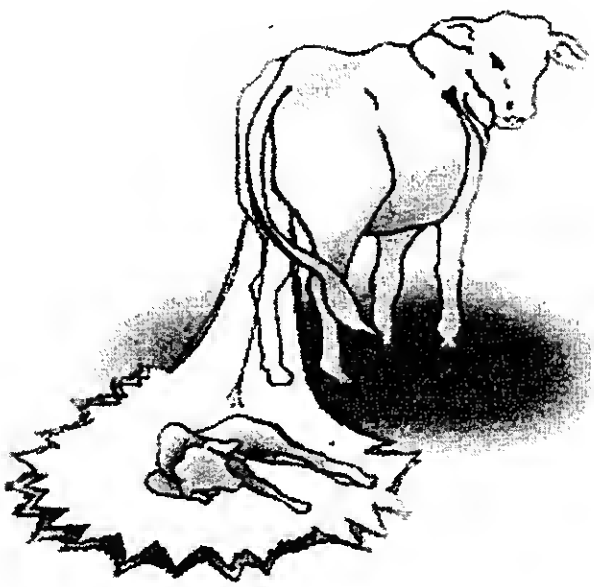
Gram -Ve Bacteria

(1)

Genus: Brucella

(مرض الاجهاض المعدي في الأبقار)



أ.د/ جمال يونس

(3) Phylum(Division):

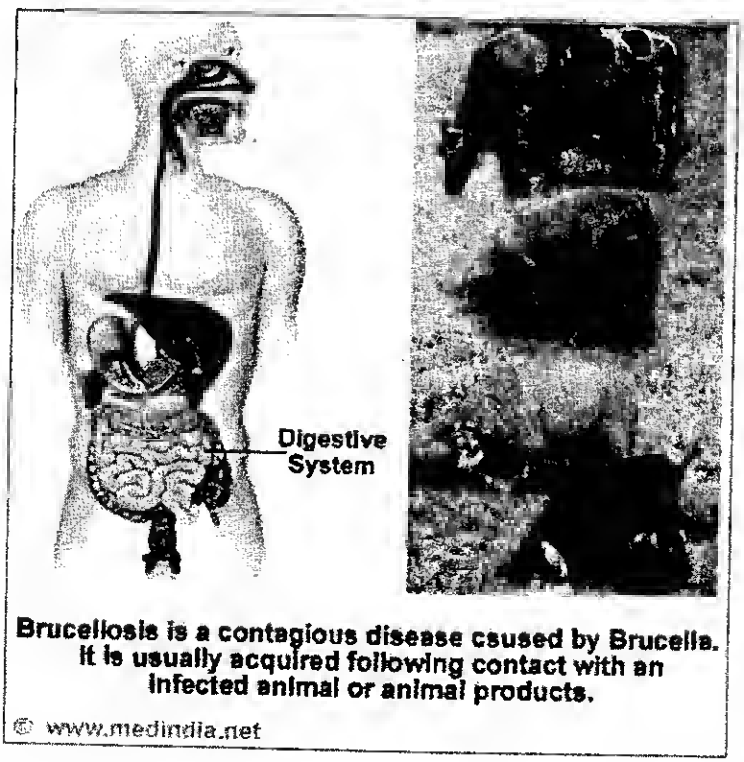
Proteobacteria

Class: α- Proteobacteria

Order: Rhizobiales

Family: brucellaceae

Genus: **Brucella**



Genus: Brucella

Species :

① B. abortus ⟶ Cattle
② B. melitensis ⟶ sheep and goats
③ B. suis ⟶ pigs
④ B. neotomae ⟶ desert Wood rat
⑤ B. ovis ⟶ Sheep
⑥ B. Canis ⟶ dogs

The 3 principal (main) brucella species

- The first 4 species are smooth strains.
  The Last 2 species are Rough strains.

- Smoothness and Roughness of brucella ⟶ depend on the presence of surface antigens (A, M and R) (Lps) on the cell wall of brucella
  - ⟶ A and M antigens (smooth strains)
  - ⟶ R antigen (Rough strains)

pathogenicity :

- Brucellae are obligate pathogens and facultative intracellular parasites ⟶ where they are phagocytosed and remain viable inside phagocytes through the prevention of fusion of Lysosomes with phagosomes.

- Cause **brucellosis (Contagious abortion, Bang's disease)** in ♀ cattle, sheep and goats → ch' by:

1- **storm of abortion** (80-90%) esp. at the Late stage of pregnancy.
2- Retention of placenta

- **♂ animals:**

1- **Infertility**
2- epididymitis, orchitis, testicular abscess
3- hygroma of joints (arthritis)
4- B. ovis → Cause **Ram epididymitis.**

- **Human:**

It is **a zoonotic disease.**

1- Cause → **Malta fever** (B. melitensis)
→ **undulant fever** (B. abortus, B. suis, B. Canis)
2- Man can be infected by all brucella species except B. ovis and B. neotomae.
3- No abortion or osteomyelitis in man.
4- Human in close contact with infected animals (slaughterhouse workers, vets, farmers and dairy workers) are at risk of developing undulant fever **(occupational disease)**

- The prediLection sites of bruceLLae are the reproductive tracts of ♂ and ♀ esp. the pregnant uterus → beCause erythritoL (which is essential for the growth of most bruCeLLae) is present in the pLaCenta and ♂ genitaL tract of CattLe, sheep, goats and pigs except humans.

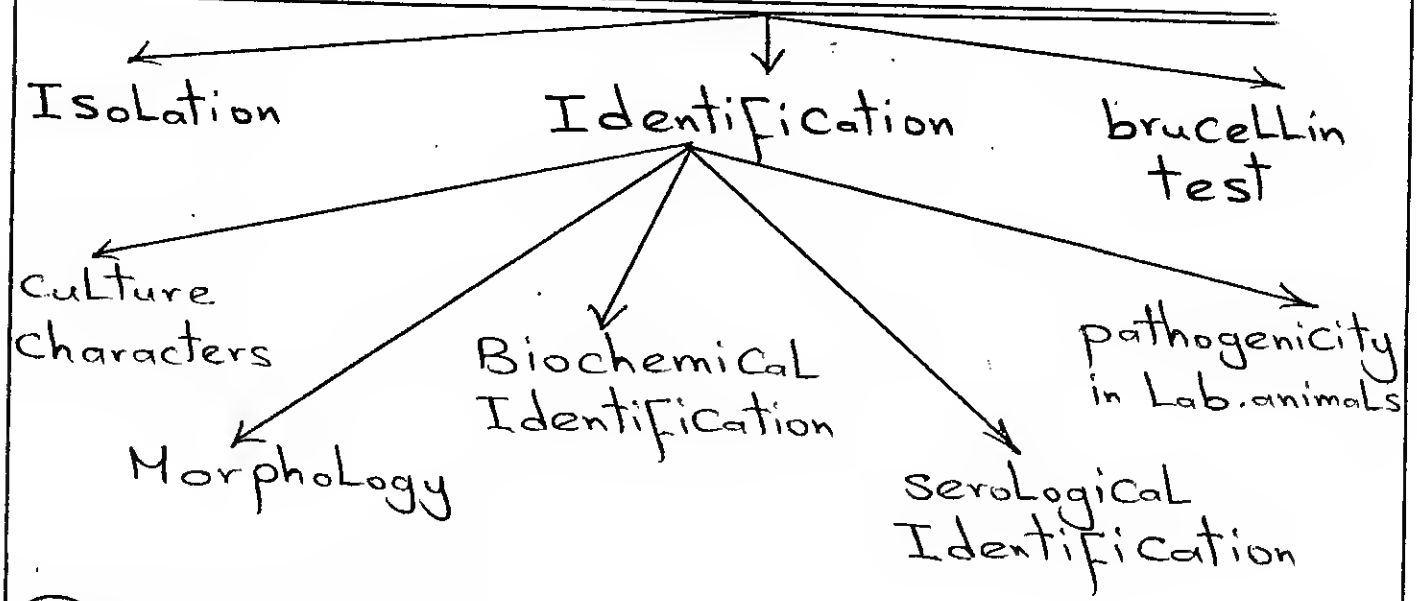
- FemaLes usuaLLy abort onLy onCe, after which a degree of immunity deveLops and the animaLs beCome **Carrier** → excrete Large No. of bruceLLae in fetaL fLuids at subsequent parturitions.

Laboratory diagnosis:

- **Specimens:**

1- FetaL membranes
2- FetaL stomach Contents
3- InternaL organs (Liver and spLeen) and Lns
4- uterine exudate of reCentLy aborted animaL.
5- Semen, testiCLes and preputiaL washings
6- abSCesses and other ext. LesionS (e.g hygroma of joints)
7- MiLK
8- Serum of Suspected animaLS for SeroLogiCaL tests.

Laboratory diagnosis of brucella :



(A) Isolation :

- O_2 requirement ⟶ aerobic except B. abortus requires 5 - 10 % Co_2 to grow (Capnophilic)
- opt. temp. ⟶ 35 - 37 °C
- pH of media ⟶ slightly acidic (pH 6.6 - 6.8)
- Incubation time ⟶ 48 hrs - 4 weeks
- Brucellae grow slowly on ordinary media (fastidious organism) ⟶ Their growth is improved by the addition of blood, serum or tissue extracts.
- Brucellae are unable to grow on MacConkey's agar.

- The general (basal) media used for the isolation of brucellae are:

Liquid media	Solid media
① Serum dextrose tryptone soya broth (SDB)	① Serum dextrose agar (SDA)
② Albimi brucella broth (ABB)	② Liver infusion agar
	③ Albimi brucella agar (ABA)

- The basal media become selective for the isolation of Brucella by addition of:

① antibiotics and antifungal agents → such as polymyxin B, bacitracin, actidione and circulin.

② dyes → such as thionin, basic fuchsin, crystal violet and pyronin.

→ to inhibit the growth of other M.o

Ⓑ Identification:

1- Culture characters:

Smooth colonies	Rough colonies
small, convex, round, grayish and translucent which become opaque and brownish with age	dull, opaque, yellowish and granular with irregular border.

- No hemolysis on blood agar.

2- Morphology:

- **stain used :**
  - → Gram's stain
  - → Modified Ziehl-Neelsen's stain
  - → specific stains ⟶ such as Kazlovski stain or Koster's stain.

- **staining reaction :**

1- Gram's stain ⟶ Gram -ve

2- Modified Z.N ⟶ Brucellae are **non-acid fast organisms** but they resist the decolourization by weak acids (such as 0.5 % acetic acid) ⟶ So, they are stained red and other organisms are stained blue ⟶ So, they are **weak acid fast organisms.**

Q: Why Brucella is weak acid fast organisms?

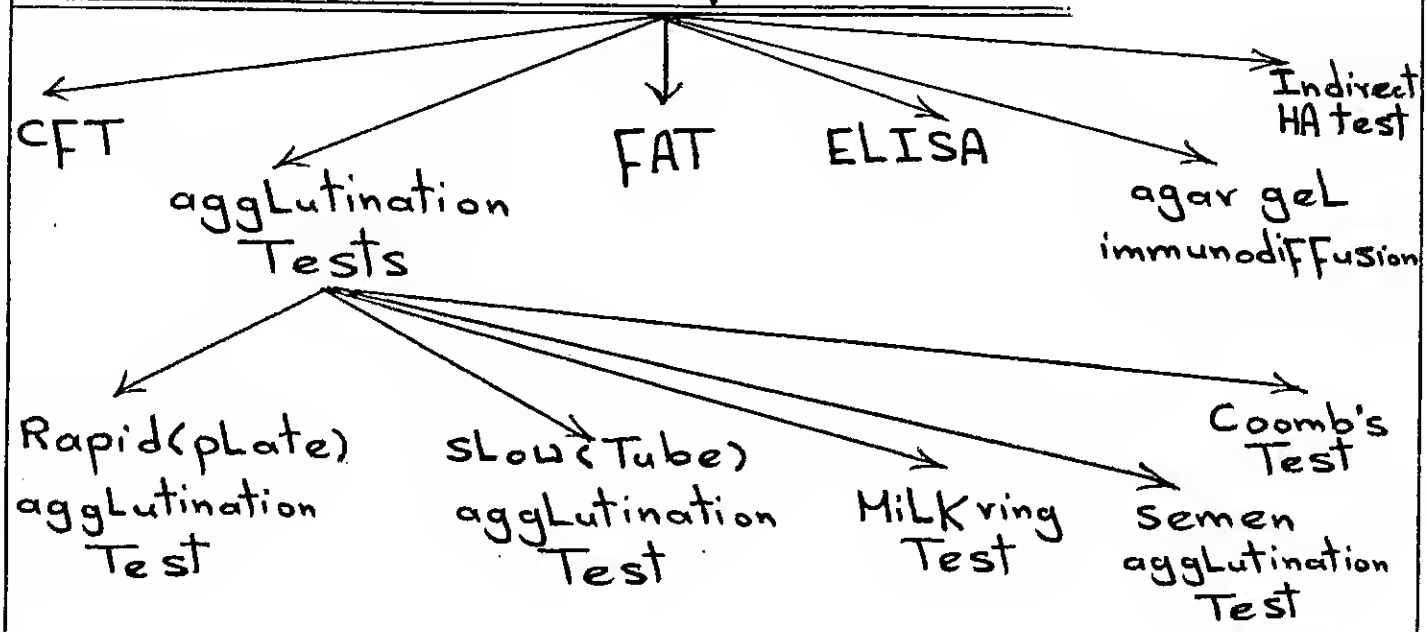
because they resist decolourization by weak acids (such as 0.5 % acetic acid) ⟶ So, they are stained red and other organisms are stained blue by modified Ziehl-Neelsen's stain.

- **shape :** Coccobacilli (pleomorphic)
- **size :** small
- **arrangement:** single, pairs or in short chains.
- Non-sporulated
- Non-capsulated
- Non-motile

3- Biochemical identification:

- Catalase +ve except B.ovis and B.neotomae
- oxidase +ve except B.ovis and B.neotomae
- Less active in Carbohydrate fermentation.

4- Serological identification:



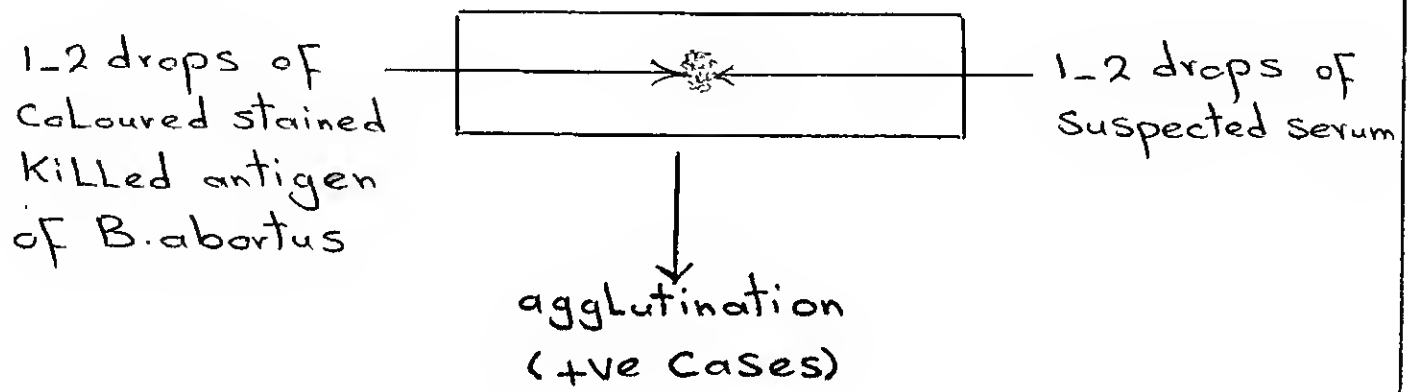
(1) Complement fixation test (CFT):

- It is highly specific and very sensitive depending on IgM and IgG_1
- Complement fixing antibodies appear in the blood of infected animals shortly before agglutinins.

② Agglutination tests :

a- Rapid (plate) agglutination test :

1- Coloured stained Killed antigen of B. abortus :
used for Control of disease and depends on IgM and IgG_1



2- Rose-Bengal plate test :

- antigens → buffered to pH 3.65 - 4 → stained with Rose-Bengal stain (Red)
- Serum → diluted with acid buffer (pH 3.5) to destroy IgM (non-specific antibodies) Leaving IgG (specific antibodies) → So, this test is more accurate.

3- Rivanol test :
depends on precipitation of IgM (mostly produced by adult Vaccination) by treating the serum with Rivanol Solution.

b- Slow (Tube) agglutination test :

1- ordinary tube agglutination test :

- It is a standard test used for animals positive for plate agglutination test.
- used routinely in the farms.
- depends on IgM and IgG_2
- done by making 2-fold dilutions of serum

Result:

	Cattle	sheep	Human
Suspecious	1/20	1/40	1/80
positive	1/40	1/80	1/160

→ Suspecious cases are re-tested after 10-15 days because agglutinins appear in the blood 10 days post-abortion.

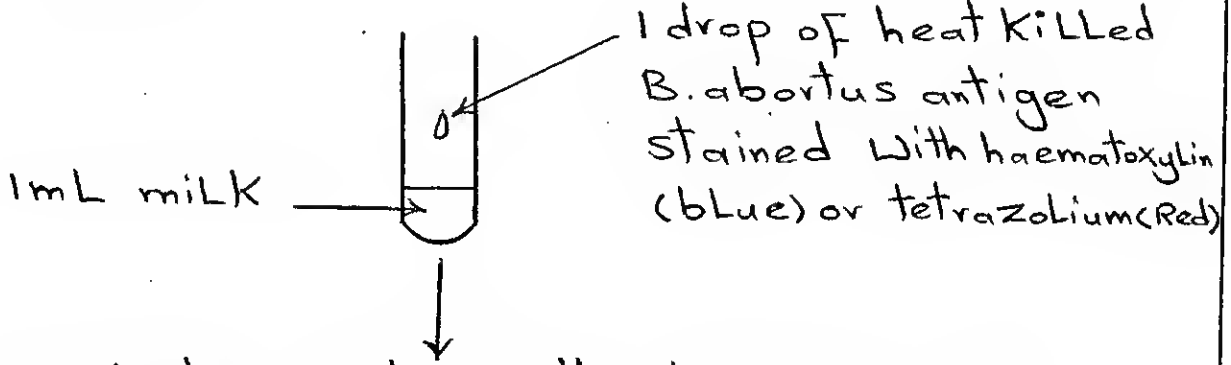
2- Mercaptoethanol test :

In which, IgM antibodies are destroyed
(These antibodies are mostly produced by adult vaccination)

c_Milk ring test
(Abortus Bang Ring test, ABR)

- depends on the presence of IgM, IgG, and IgA
- This test depends on that the agglutinins which are excreted in milk during infection are carried to the surface with the rising fat globules.

- **procedure:**



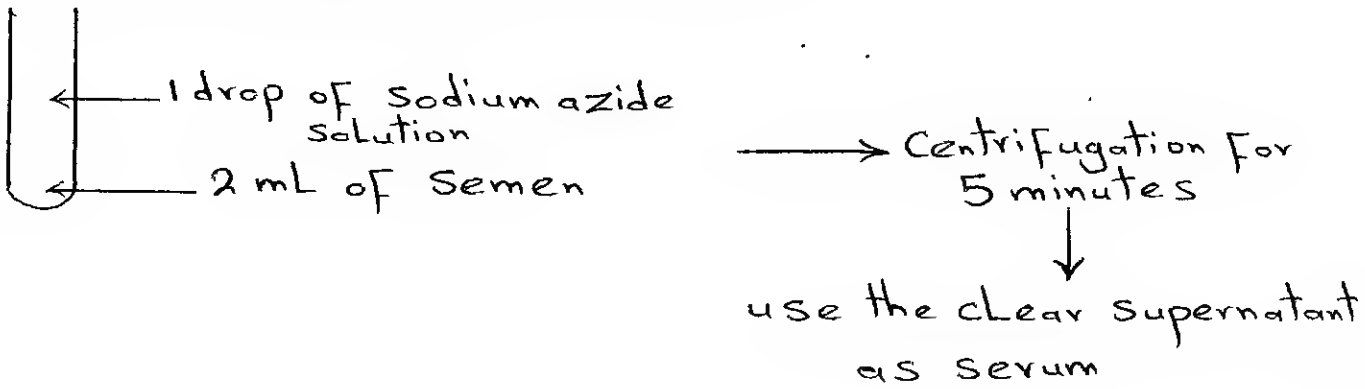
Incubate in water bath at 37°C for 1 hr

- **Result:**

Cream Layer	Coloured	uncoloured	Coloured
Milk	uncoloured	Coloured	Coloured
Result	+Ve	-Ve	Suspecious

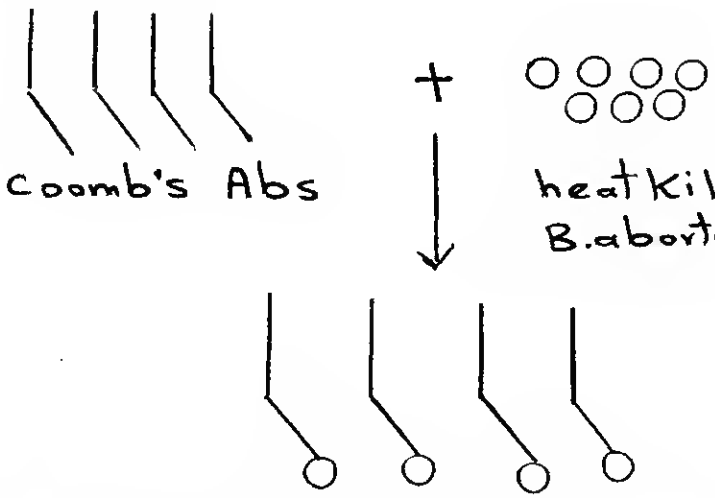
→ Suspecious cases are retested after 10_15 days

d- Semen agglutination test :

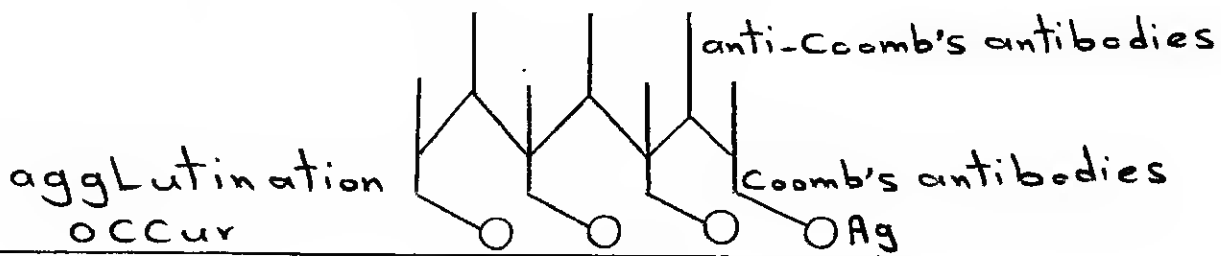


e- Coomb's test :

Very sensitive in chronic infection → due to the production of Coomb's antibodies (Incomplete antibodies contain one Fab portion only)



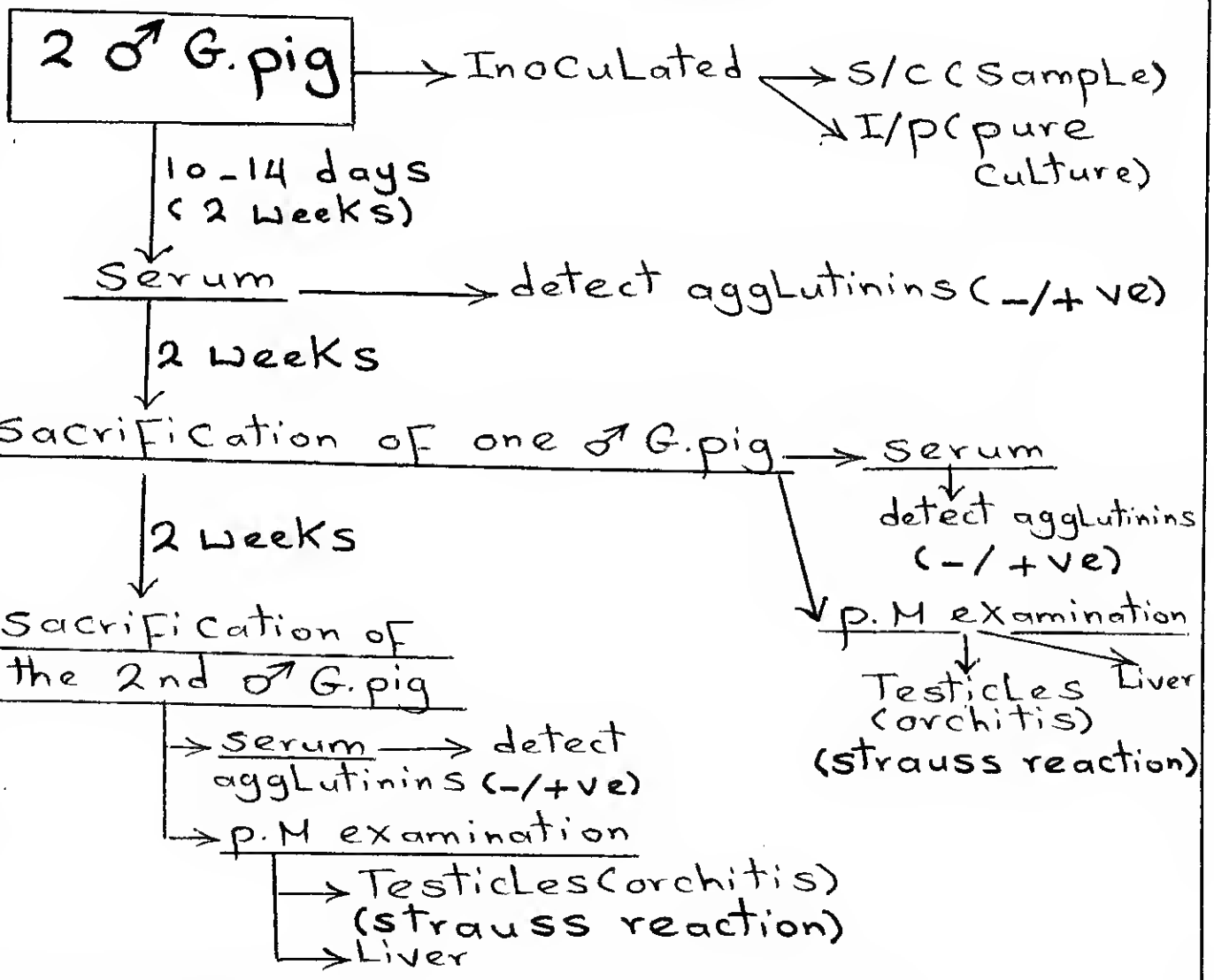
So, use anti-Coomb's antibodies (antiglobulins)



5-pathogenicity in Lab. animals:

→ It is used for diagnosis as well as for getting a pure culture of the organism.

→ Blood samples should be taken from ♂ G.pigs before inoculation and tested for the presence of agglutinins.



→ Isolation of M.o in a pure culture

N.B:

- The sample is inoculated s/c because it is very highly contaminated ⟶ where it is filtrated in Ln ⟶ followed by M.o proliferation

- Bacteria causing strauss reaction:
  - → Brucella
  - → Burkholderia mallei
  - → Corynebacterium ovis (C.ovis)

© Brucellin test:

It is allergic skin test depends on delayed type of hypersensitivity.

- In suspicious cases to be infected with brucella, I/D injection of brucellin (specific protein extract of the organism) ⟶ result in activation of sensitized T-Lymphocytes to secrete Lymphokines (Cytokines) which are responsible for skin reaction.

1- chemotactic factor ⟶ attraction of macrophage and uncomitted (unsensitized) Lymphocytes to site of injection.

2- MIF ⟶ inhibit migration of macrophage

3- γ-IFN ⟶ activate macrophage

4- Macrophage aggregation Factor (MAF) ⟶ giant cell formation (chronic disease)

5- skin permeability factor ⟶ ↑ capillary permeability

+ve reaction:

oedema then Induration (measured by caliper)

- It is satisfactory for diagnosis of Malta fever in human but unreliable in cattle.

dr. M. abdel na3eem
(B.vsc , M.vsc , ph.D)
01003912810

differentiation of brucella species

a- serotyping of brucella:

→ there are 2 surface antigenic determinants (A and M) shared among the 3 principal brucella species ⟶ so, they are responsible for the cross reactions (i.e antisera from immunized animals with one smooth strain will agglutinate the 3 principal brucella species)

→ these surface antigens of brucella are determined by the composition of LPS complexes in the cell wall.

A
main antigen of B. abortus and B. suis

M
main antigen of B. melitensis

R
main antigen of non-Smooth (Rough) species (B. ovis, B. Canis)

→ antigenic A : M ratio :

3 principal brucella spp.	A	M
1- B. abortus and B. suis	20	: 1
2- B. melitensis	1	: 17.5

→ Serotyping of brucella is carried out either by slide or tube agglutination test using monospecific A, M and R antisera.

→ Common Ag of brucellae (LPS antigen) cross react with E. coli, salmonellae, pseudomonas spp., Campylobacter spp. and Vibrio cholerae.

b- differential characteristics of the three principal brucella species:

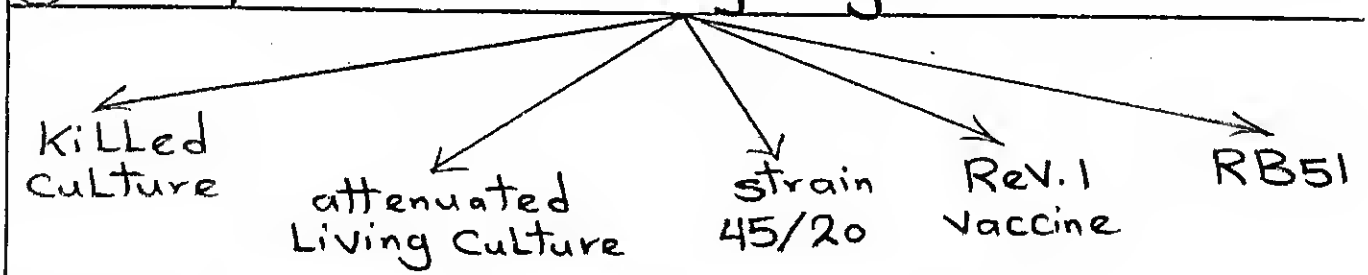
points of differences	B. abortus	B. melitensis	B. suis
1- Co_2 requirement	+	−	−
2- H_2S production	+ (after 2 days)	−	+ (after 4 days)
3- Hydrolysis of urea	slow (1-2 hrs)	variable reaction	Rapid (within ½ hr)
4- growth on dye containing media:			
• Thionin 1/50,000	−	+	+
• Methyl violet 1/50,000	+	+	−
• Basic Fuchsin 1/50,000	(+)	+	(−)
• pyronin 1/100,000	+	+	−
5- agglutination using monospecific antisera:			
• A (abortus)	(+)	(−)	(+)
• M (melitensis)	(−)	(+)	(−)

Immunity
(Immunization or vaccination against brucella)

(a) Natural Immunity:

→ young animals are more resistant to infection than adult ones due to presence of maternal immunity.
→ Recovered animals develop state of immunity but still excrete the organism in their discharge.

(b) artificial immunity by vaccination:



1_Killed Culture (Bacterin):

Killed Culture of B. abortus either by heat or chemicals with oil adjuvant.
→ It is not harmful and animals are vaccinated twice by it.
→ produce neither solid nor Lasting immunity because it produce humeral immunity but not cell mediated immunity (which is protective)
→ produce complement fixing antibodies.

2- attenuated Living Culture:

→ B.abortus strain 19 (Book strain) ⟶ used for immunization of cattle at age of 6-8 months old ⟶ S/C (single injection)

- animals ↓ 5 months old should not be vaccinated:

1- to avoid the interference with maternal immunity.
2- cells and tissues of the immune system will not be so highly developed to form a level of Abs sufficient to produce good protection.

- disadvantages:

① adult animals develop permanent agglutinins which interfere with agglutination test results ⟶ so, it is difficult to differentiate between the diseased one and the vaccinated one.
② may cause severe postvaccination reaction ⟶ abortion in pregnant cattle.
③ Contraindicated in vaccination of ♂ at any age
- because it produce permanent agglutinin and so can't differentiate between the infected male and vaccinated one.
- may cause infertility in some ♂ calves

3- strain 45/20 :

It is rough strain of B.abortus.

→ the strain No. 45 is subcultured 20 times.

disadv.:

Live rough strain of B.abortus 45/20 is virulent in vivo.

4- Rev.1 vaccine:

→ prepared From B.melitensis

→ used For vaccination of sheep and goats

→ given S/C

5- RB51 :

It is rough strain of B.abortus

Q: Which is better For vaccination against brucella, Killed Culture or strain 19?

1- strain 19 is more preferred ⟶ because it produce cell mediated immunity which is protective against brucella.

2- While Killed Culture produce humeral immunity which is not protective.

• Immunity in brucellosis is predominantly cell-mediated.